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ON A PARASITE FOUND IN THE WHITE CORPUSCLES OF THE
BLOOD OF DOGS.

BY

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ON A PARASITE FOUND IN THE WHITE CORPUSCLES OF THE BLOOD OF DOGS.

SO far as I am aware there is no record in literature of the discovery of parasites of the white blood corpuscles of mammals. For this reason I am led to record some observations I have made upon a leucocytic parasite which is not uncommon in the peripheral blood of dogs in Assam.

Although I do not consider that the parasite I am about to describe has any connection with the leucocytic parasites of birds described by Danilewsky, Sacharoff, Ziemann, and others, it will not be out of place if I refer briefly to the work of those observers, for parasites of the leucocytes have been discovered so rarely that our knowledge regarding them is very slight.

Danilewsky¹ discovered a parasite in the white blood corpuscles of a screech-owl between 1884 and 1886. He studied it only in fresh preparations. In his first communication he described it as a spherical or oval lightly granular body contained in a delicate transparent homogeneous envelope provided with a large biscuit-shaped nucleus. He did not describe a nucleus as being present in the parasite itself and evidently was interested chiefly in the nature of the mass in which the parasite was contained. From the size and shape of this envelope, the absence of grains of melanin in it, and the appearance and size of its nucleus he concluded that it was a white blood corpuscle—that the parasites were, in fact, *Leucocytozoa*. He observed frequently that the body-substance of the leucocytes in which the parasites were contained was drawn out in the form of triangular tags and for this reason concluded that the leucocytes were in a state of degeneration. Each parasite was described as being more than $1\frac{1}{2}$ times as large as a red blood corpuscle.

In his second communication, published in 1890, he described the cells containing parasites as having the appearance of large fusiform bodies with a central granular portion and an elongated excentrically situated nucleus. The granular mass was sometimes spherical in shape. He repeated his opinion that the parasite was contained, not in a red blood corpuscle, but in a degenerated leucocyte, and stated that he had been able to show that it was only an intracellular stage in the development of the flagella bodies (*Polimitus*) previously described by him as being present in the blood of birds. He considered, however, that the flagella bodies produced from the leucocytozoon differed from those which develop in red blood corpuscles in being of larger size and in having no granules of melanin in their interior. In the bone-marrow he observed young stages of the parasite as small oval or spherical bodies enveloped by an

almost homogeneous layer of leucocytic protoplasm which had lost the power of amœboid movement. The nucleus of this envelope was large and distinct and almost always much elongated; if the parasite was of considerable size it pressed upon the nucleus and caused it to become biscuit-shaped. In attempting to explain why the parasites are not destroyed by the phagocytic action of the cells in which they are contained he threw out the suggestion that possibly these cells are not, after all, fully developed leucocytes but erythroblasts.*

In a third paper he described in more detail the development of flagella bodies from a leucocytozoon found in the blood of a grey crow. In this case the parasite was described as a large round finely granular body containing a small clear nucleus.

Sacharoff² described parasites of the leucocytes as being present in the blood of crows, rooks, and magpies. In crows they were present along with parasites of the red blood corpuscles. He examined them both in fresh preparations and in preparations stained by Romanowsky's method. In fresh preparations a nucleus could not be seen in the parasites, but, after staining, a faint rose-coloured nucleus with an indistinct contour could be made out. He described two forms as being present in the blood of crows, *viz.*, (1) forms containing numerous deeply staining granulations and some vacuoles; and (2) homogeneous forms. Flagella bodies were frequently observed to originate from the homogeneous parasites, but very rarely from the granular ones. He considered that the parasites of the blood of crows pressed upon and gradually destroyed the nuclei of the leucocytes in which they were contained and for this reason regarded them as a form of *Karyophagus*. He described the youngest forms as being found in the blood of rooks. They were oval or fusiform bodies of the size of an eosinophil granule, and were usually present in lymphocytes, but sometimes free in the plasma. They possessed a small nucleus. He considered that the parasites found in rooks differed from those found in crows in that they did not destroy the nuclei of the cells in which they were contained.

In 1898 Ziemann³ discovered similar leucocytic parasites in the blood of three owls. He examined them chiefly in preparations stained by a modification of Romanowsky's stain. He described three forms, *viz.*, (1) round or oval sharply outlined parasites from $\frac{2}{3}$ to the full size of a red blood corpuscle. These were free in the plasma. In stained preparations they presented a compact round or elongated nucleus, and in their body protoplasm a stained outer zone and an unstained, or less deeply stained, inner zone could nearly

* The word used by Danilewsky is *hématoblaste*; it must not be confounded with the English word *hæmatoblast*. For definition of the latter term and of erythroblast see Quain's *Anatomy*, 10th edition, vol. I, part II, pages 215 and 219.

always be made out ; (2) forms resembling these but enclosed in a sharply outlined mass (leucocyte) possessing numerous small granulations. The body of this form stained deeply and contained dark-staining granules and light unstained spots. Its nucleus was round and stained deeply. The leucocyte in which the parasite was contained was often shaped like a whetstone and occasionally reached a length of 44 micromillimetres : its nucleus was dumb-bell-shaped and partially encircled the parasite ; (3) round forms two or three times as large as the previous form with larger and more numerous clear spots.

Ziemann did not observe multiplication stages of any kind and in the internal organs of two owls upon which *post-mortem* examinations were made the parasites presented the same appearances as in the peripheral blood. He regarded the parasites found by him as different from those found by Danilewsky.

Berestneff⁴ studied leucocytic parasites in an owl, a crow, and a magpie. In the owl and crow they were present together with *Halteridium danilewskyi* and in the crow filariæ also were present. In the magpie no other parasites were seen. In fresh preparations of the owl's blood the leucocytozoon was present in long spindle-shaped and round forms. The spindle-shaped forms contained numerous small round granules and a clear round nucleus ; the round forms had the appearance of a large ring containing dull protoplasm in which an oval nucleus was seen. Almost the whole of the latter form of the parasite was encircled by the nucleus of the leucocyte. In preparations stained by Romanowsky's method the body of the spindle-shaped forms stained intensely blue and contained small deep violet-coloured granules and unstained round spots. The nucleus was usually oval in shape and only faintly stained : near it lay a deeply coloured round or irregularly shaped "nucleolus" of about .7 to 1.5 micromillimetres in diameter. The second form was about the length of the diameter of a red blood corpuscle. Its body-substance stained faintly and was homogeneous or only slightly granular. The nucleus was considerably larger than that of the first form. The nucleus of the leucocyte was closely applied to the parasite and often was of an irregularly quadrilateral shape.

Young stages of the second form of parasite were seen by Berestneff in mononuclear leucocytes (large lymphocytes). Their protoplasm was faintly stained and contained a large nucleus and some deep blue granules. He regarded both types of the parasite seen by him as sexual forms, those with feebly staining body-substance and a large nucleus being the males, and those with deeply staining body-substance, a small nucleus, and a "nucleolus," being the females. He was unable to confirm the observations of previous workers regarding the development of flagella bodies from the parasites.

Laveran⁵ and Schaudinn⁶ have also studied these leucocytozoa in the blood of owls. Laveran described a female form and an immature male form observed

in the blood of an owl, *Syrnium aluco*. Neither form contained pigment. In opposition to Ziemann and other observers he regarded the elongated, spindle-shaped elements in which the parasites were included, not as leucocytes, but as deformed red blood corpuscles with hypertrophied nuclei. He considered that the male and female gametes carried out fertilization in the same manner as members of the genera *Plasmodium* and *Halteridium* and therefore regarded the parasites as *Hæmamoeba*, naming them *Hæmamoeba ziemanni*.

Before referring to Schaudinn's work upon these parasites it will be well to summarize in the following brief manner the somewhat contradictory results outlined above. In addition to the well-known pigmented parasites *Halteridium* and *Proteosoma*, there have been found in the blood of certain birds unpigmented nucleated parasites which may be present in two forms, *viz.*, (1) a deeply staining granular (female) form containing a "nucleolus" as well as a nucleus; and (2) a feebly staining homogeneous (male) form. In the peripheral blood of the birds both these forms usually have been observed to be contained in, or attached to, elongated nucleated elements which Danilewsky, Sacharoff, Ziemann, and Berestneff regarded as leucocytes, but which Laveran thought were deformed red blood corpuscles. Danilewsky, however, in one of his papers, threw out the suggestion that some of the elements in which the parasites were contained were possibly erythroblasts, and Sacharoff, as regards the cells in which the parasites of the blood of rooks (but not of crows) were contained, thought this suggestion very probably correct. Several of the observers described the production of flagella (microgametes) from one or other form of the parasite when the blood was examined in fresh preparations. Danilewsky termed the parasites *Leucocytozoa*; Sacharoff and Berestneff looked upon them as being probably a form of *Karyophagus*; and Laveran considered that they were true *Hæmamoeba*.

At this stage of knowledge Schaudinn commenced his study of these parasites. He observed the two forms seen by previous investigators in the peripheral blood of owls and confirmed the correctness of Laveran's opinion that they are male and female sexual forms which, after their exit from the body of the bird, undergo development in the same manner as parasites of the genus *Plasmodium*, *i.e.*, by the production from the male form of flagella (microgametes) which fertilize the female form or macrogamete, the resulting body being an ookinet. He recorded the following additional facts as a result of his researches. The formation of the microgametes and the fertilization by them of the macrogametes normally takes place in the mid-gut of a "culex" mosquito. The ookinet which results becomes greatly elongated and its nucleus divides up into a large number of smaller nuclei each of which becomes surrounded by a dense zone of plasma. From the cellular areas thus formed there are developed a number of trypanosome-like flagellates which separate themselves from the

residual body, become much elongated, and develop into typical spirilla. These spirilla migrate into the malpighian tubes of the mosquito and undergo multiplication by longitudinal division. They are to be considered as having periods of rest and periods of movement. During the former they enter the cells of the malpighian tubes; during the latter they become free, multiply by longitudinal division, and after having flooded the malpighian tubes reach the anterior part of the oesophagus of the mosquito whence they are again transferred by means of the bite of the mosquito into the blood of the first host. Here they undergo asexual multiplication in the form of spirilla and are ultimately transformed into the sexual stages (the so-called leucocytozoa) from which their cycle of development commenced. In the body of the bird as in that of the mosquito they are to be regarded as having periods of rest and periods of movement. In the blood of the spleen and bone-marrow such of the flagellates (spirilla) as are to become sexual forms undergo a remarkable increase in size and attach themselves by their posterior extremities to certain elements which Schaudinn considers to be erythroblasts. They then pass into a period of rest and gradually absorb into their substance the body of the erythroblast. At this stage they may appear in the peripheral blood, and it is in this condition that they were discovered by Danilewsky and others. After remaining in the stage of rest for a certain time the flagellate apparatus is formed anew, the nucleus of the erythroblast is cast off, and the parasite enters again upon a period of movement in the form of a trypanosome-like flagellate. The periods of rest and of movement alternate. Growth takes place during the former, multiplication during the latter.

The so-called leucocytozoa discovered by Danilewsky and studied by Sacharoff, Ziemann, and others are, therefore, according to Schaudinn, the resting sexual stages of his trypanosome-like parasite, the nucleated mass in which those observers thought the parasite was contained being, in his opinion, neither a leucocyte nor a red blood corpuscle but an erythroblast to which the parasite has attached itself and which (with the exception of the nucleus) it absorbs into its own substance. The "nucleolus" observed by Sacharoff, Berestneff, and Laveran in one of the forms is considered by Schaudinn to be a true blepharoplast.

The cycle of development undergone by these so-called leucocytozoa is therefore, according to Schaudinn, very similar to that of *Halteridium* except that, while the resting stage of the latter parasite is passed at the expense of the red blood corpuscles that of the former is passed at the expense of those forms of white cells known as erythroblasts. For this reason their development, while in the bodies of the birds, is carried on chiefly in the bone-marrow. In other ways the details of development of the leucocytozoa are somewhat different from that of the halteridia, and Schaudinn regards them as belonging, not to the genus

Trypanosoma, but to the genus *Spirillum*, and names them *Spirillum siemanni*.

Ziemann⁷ now agrees with Schaudinn in regarding the so-called leucocytozoa found in the peripheral blood of owls as stages in the development of a trypanosome-like organism; and Edmond and Etienne Sergent⁸ have partially confirmed Schaudinn's observations.

So far as I can ascertain, the above is a brief summary of all that is known regarding parasites of leucocytic blood cells, and now I shall describe the parasite I have studied in the blood of dogs. Observations upon this organism are simplified by the fact that there can exist no doubt regarding the nature of the anatomical element of the blood in which it is contained.

In June 1904 Dr. C. A. Bentley, of Borjulie, Assam, informed me that he had found a peculiar body in the leucocytes of the blood of a dog. He thought the body might be a parasite of some kind, but, with the stain he was then using he had been unable to bring out any details of structure in it. He showed me a slide in which some of the leucocytes contained a body having the appearance of a large regularly shaped vacuole. In a few of the bodies a very faintly stained nucleus could be seen. Fortunately Dr. Bentley knew the owner of the dog in the blood of which the bodies had been found, and the next day we were able to procure more slides from it and to study the parasite together for some days. Afterwards I made a practice of examining the blood of such dogs as I was able to obtain in the places I visited, with the following results:—

Place.	Number of dogs examined.	Number in which the parasite of the leucocytes was found.	Remarks on the presence or absence of other parasites.
Borjulie	16	2	One dog in which the leucocytic parasites were not found had filarial embryos in its blood.
Gauhati	3	1	<i>Piroplasma canis</i> was also present at one period of this case.
Tezpur	5	2	In one of the two cases filarial embryos were also present.
Silghat	7	0
Nowgong	8	1	No other parasites present.
Dacca (Bengal) . .	6	0

I have therefore found the parasite in the peripheral blood of six out of 45 dogs examined; in four cases it was present alone, in one case it was present together with filarial embryos, and in one case with parasites of the red blood corpuscles (*Piroplasma canis*).

The number of parasites present in films of the peripheral blood varied

greatly in the different cases. The following results of differential counts of the leucocytes made in four cases will show this, as well as the relative proportions of the different varieties of leucocytes present :—

Case 1.—Number of leucocytes enumerated = 600.

Number of parasites seen = 35.

Relative proportions of the different varieties of leucocytes:—

Polymorphonuclear cells	67 per cent.
Large mononuclear cells	3 per cent.
Small mononuclear cells	29 per cent.
Eosinophil cells	1 per cent.

Case 2.—Number of leucocytes enumerated = 1,165.

Number of parasites seen = 58.

Relative proportions of the different cells:—

Polymorphonuclear cells	58·2 per cent.
Large mononuclear cells	4·1 per cent.
Small mononuclear cells	36·4 per cent.
Eosinophil cells	1·1 per cent.

Case 3.—Number of leucocytes enumerated = 500.

Number of parasites seen = 16.

Relative proportions of the different cells:—

Polymorphonuclear cells	65 per cent.
Large mononuclear cells	3·4 per cent.
Small mononuclear cells	30·2 per cent.
Eosinophil cells	1·4 per cent.

Case 4.—In this case filarial embryos were also present.

Number of leucocytes enumerated = 590.

Number of parasites seen = 6.

Relative proportions of the different cells :—

Polymorphonuclear cells	65·7 per cent.
Large mononuclear cells	2·8 per cent.
Small mononuclear cells	22·4 per cent.
Eosinophil cells	8·9 per cent.

In the first and second cases one parasite was seen to about every 11 polymorphonuclear cells, in the third case one parasite to about every 20 polymorphonuclear cells, and in the fourth case one parasite to about every 64 polymorphonuclear cells.

A special feature of all the films was the presence of nucleated red blood corpuscles in considerable numbers.

I have had but few opportunities of examining the parasites in fresh preparations, and I shall therefore confine myself chiefly to a description of them as they appear in films fixed in alcohol and stained by Romanowsky's method.

In the first place it should be mentioned that in films quickly made in the ordinary way from a drop of blood removed from the foot or ear the parasites are almost invariably contained in polymorphonuclear leucocytes. During the

examination of 2,855 leucocytes in films from the four cases mentioned above as well as during less minute examinations of slides from two other cases, I have seen only one parasite which was free in the plasma; every other was contained in a polymorphonuclear cell.

In the second place it is necessary to mention that in order to bring out the structure of the parasites distinctly the films must be well and deeply stained. When this has been done the parasites are seen very clearly and cannot easily be passed over during the routine examination of a slide. As will be seen from the drawings at the end of this paper the chief characteristic of the bodies as they present themselves during the examination of a stained film is their remarkable uniformity in size and appearance. They are usually about $1\frac{1}{2}$ times as long as the diameter of a red blood corpuscle and the majority are almost exactly twice as long as they are broad. The shape of the body may perhaps be best compared to that of a bean.

On careful examination of a well-stained film it will be seen that these characteristic bean-shaped bodies present in the leucocytes consist of two parts, *viz.*, (1) a definite oblong almost quadrilateral capsule or *cytocyte*, the outline of which is often coloured bright pink with Romanowsky's stain; and (2) a parasite enclosed within this capsule.

The capsule or cytocyte would appear to be formed from the protoplasm of the leucocyte as the result of the presence of the parasite. It is thick and evidently resists considerably the penetration of the stain through it, for unless prolonged staining has been employed, the parasite itself remains uncoloured and the appearance presented is like that of the body shown in one of the leucocytes in figure 7. The sides of the capsule are often pressed inwards and folded over so that in stained films they appear as in figure 10.

The parasite itself is inside the capsule. This it often completely fills and in such cases its outline cannot be clearly differentiated from the outline of that structure (Figs. 1, 2, 3, 4, etc.). In many cases, however, it is somewhat retracted or shrunken so that there is a clear space between its circumference and that of the capsule (Fig. 5). In these cases its shape and characteristics are clearly seen. It is an oblong bean-shaped body composed of a homogeneous or very slightly granular protoplasmic body provided with a large oval or round chromatin nucleus. Its body-substance stains a faint sparrow-egg blue colour and its nucleus, which is usually situated near one end, stains in the same manner as the nuclei of the leucocytes. The parasite has a clearly defined circumference but apparently is not surrounded by a definite membrane, and its appearance is such as to indicate that when not imprisoned within its leucocytic capsule, it has the power of movement. It contains no grains of pigment.

The great majority of the parasites seen in a stained film will be found to correspond with the above description; they are oblong, nucleated, unpigmented bodies contained in a definite bean-shaped capsule or *cytocyte* apparently formed from the body-substance of the polymorphonuclear leucocyte in which the parasite is situated.

By a careful examination of a large number of parasites the following additional facts may be made out.

1. *As regards the position of the parasite in the leucocytes and its effect upon them.*—The organism is situated well within the body-protoplasm of the leucocyte and, nearly always, there is a fairly thick layer of this body-substance between it and the nucleus of the leucocyte (Figs. 1, 10, 13, etc.). Sometimes a portion of the circumference of the capsule touches the nucleus of the leucocyte, but it does not seem to press upon it, and examples are often seen in which a band of the leucocyte nucleus passes over the parasite in the manner shown in figures 2 and 3. The leucocytes appear, indeed, to be little, if at all, affected by the presence of parasites in them; they are sometimes larger than leucocytes in which no organism is present, and sometimes their body protoplasm stains somewhat more deeply, but their nuclei retain their original characteristic shape and staining properties, and I can detect no appearance suggesting degenerative changes. The examination of fresh preparations shows that they are not less amoeboid than leucocytes in which no parasite is contained.

2. *As regards the capsule surrounding the parasite.*—The leucocytic capsule or cytocyte in which the parasite is contained has sometimes the appearance of having been torn open during the preparation of the specimen. In these cases the parasite itself is more clearly seen.

3. *As regards the parasites themselves—*

(a) From a careful examination of a large number of parasites, I believe that two forms are always present in the peripheral blood of infected dogs, *viz.*: (1) those the body protoplasm of which stains uniformly blue and which contain, with the exception of the nucleus, no chromatin granules; and (2) those which, in addition to the nucleus, contain a definite faintly stained minute chromatin micro-nucleus or centrosome (Figs. 7, 8, 10). This micro-nucleus is usually situated near one or other side of the parasite at some distance from the nucleus and is surrounded by a circular area of clear unstained protoplasm (Fig. 10). Occasionally there are, in addition, a few other granules of chromatin scattered through the body of this form.

(b) In addition to the ordinary bean-shaped bodies there are usually present in films a very few parasites almost round in shape and with only a very indistinct capsule (Fig. 11). Their contour is only very indistinctly marked off from the general body-protoplasm of the leucocytes in which they are present.

It is possible that they are less mature forms than the more common encapsuled bean-shaped parasites.

(c) During the examination of stained films from six cases, I have seen only one example of a parasite free in the plasma. Its outline was regular and of the general shape of an almond, being considerably thicker at one end than at the other. The nucleus was much elongated and lay near the centre, but touching one edge, of the parasite. The micro-nucleus or centrosome was remarkably distinct.

(d) Two parasites may occasionally be present in the same leucocyte (Fig. 13). I have observed this twice during the examination of films from six cases.

4. *As regards the nucleus of the parasite.*—In by far the greater number of parasites (about 86 per cent.) the nucleus is situated near one end and is round or oval in shape. Sometimes, however, it lies at or near the centre and in such cases it is often considerably elongated (Fig. 3). In well stained specimens it nearly always contains a bright unstained spot (Figs. 6 and 9) and the chromatin network is well displayed. I have never seen the nucleus divided into two parts nor any other signs of division in it, but the appearance shown in figure 9, where the nuclei appear to be made up of a number of small round chromatin granules, several of which may project beyond the edge of the nucleus, is frequently to be observed.

In fresh preparations the parasite in the body-protoplasm of the leucocyte has the appearance of a refractile elongated body in which no definite structure can be made out.

On one occasion I was fortunate enough to observe in a fresh preparation the escape of a parasite from the leucocyte in which it was contained. The leucocyte had been under observation for a considerable time when suddenly the worm-like body, roughly depicted in figure 12, emerged, and by reason of its slow but continuous vermicular movements gradually freed itself entirely from the leucocyte, leaving in the place it had occupied a clear space as in the lower diagram of the figure. The escaped parasite was watched for an hour, but although it continued to indulge in slow vermicular movements it did not again succeed in changing its position on the slide, and throughout the period of examination remained almost touching the edge of the leucocyte. Its general shape is shown in figure 12, the most characteristic feature being the fact that it was considerably more pointed at one end than at the other.

It remains only to refer briefly to the body shown in figure 14. This was a large unpigmented obviously amœboid body found in a stained film from a case in which the parasites were very numerous. Its body-protoplasm was stained pale blue and contained two definite patches of chromatin near the centre as well

as a considerable number of scattered chromatin granules disposed throughout its substance. Whether this body is to be regarded as having any connection with the parasites under discussion I am unable to say.

I have performed no *post-mortem* examination on any animal infected with the parasite, so that my observations regarding it are limited entirely to its appearance in the peripheral blood. While it undoubtedly has affinities with the hæmogregarines the fact that it is parasitic upon the leucocytes *and that it represents a hitherto entirely unknown form of mammalian blood infection* would seem to justify its recognition as the type of a new genus. Until further researches shall have revealed its exact zoological position the name *Leucocytozoon canis* appears to me to be an appropriate one for purposes of reference.

So far as I was able to ascertain the dogs were but little, if at all, affected by the presence of the parasites. The temperature of one heavily infected dog was taken as frequently as possible during a period of ten days with the following results :—

1st day.	2nd day.	3rd day.	4th day.	5th day.
	6 a.m. 99·2	6 a.m. 98·6	6 a.m. 98·6	6 a.m. 99·6
		10 a.m. 98·6	2 p.m. 99·4	
6 p.m. 100·6	6 p.m. 99	6 p.m. 98·4	6 p.m. 100·4	
10 p.m. 100·2	10 p.m. 99	10 p.m. 98·4	10 p.m. 100·6	
6th day.	7th day.	8th day.	9th day.	10th day.
6 a.m. 99·6	6 a.m. 100	6 a.m. 98·8	6 a.m. 100·6	6 a.m. 99·8
2 p.m. 99·4	2 p.m. 100·6	2 p.m. 100	2 p.m. 101·4	
6 p.m. 99	6 p.m. 99		6 p.m. 101·8	
10 p.m. 99	10 p.m. 98·6		10 p.m. 100	

I consider that the variations above a normal temperature which occur in the above record are not greater than are often observed in perfectly healthy animals during the summer in India.

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 The article referring to the parasites under discussion is entitled *Eine neue Parasitenform beim Steinkaus—Athena noctua—(Das sogenannte Leucocytozoen Danilewskyi?)*. Ziemann's illustrations are reproduced, and a short account of the parasite is given in the article on malaria parasites by Ruge in Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen*, page 840.
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Fig 1



Fig 2



Fig 3



Fig 4



Fig 5



Fig 6



Fig 7



Fig 8



Fig 9



Fig 11

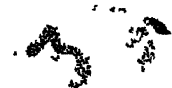


Fig 12



